

Comparative antimicrobial
studies on plant species known
as 'Pasak Bumi': *Eurycoma*
longifolia Jack., *Rennelia*
elliptica Korth. and *Trivalvaria*
macrophylla miq.

by Enih Rosamaha

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RESEARCH NOTE

Comparative antimicrobial studies on plant species known as 'Pasak Bumi': *Eurycoma longifolia* Jack., *Rennelia elliptica* Korth. and *Trivalvaria macrophylla* miq. [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Pasak Bumi is a local name for a medicinal plant in Kalimantan, Indonesia. It is a famous medicinal plant and commonly used in traditional medicine as an aphrodisiac, as well as in the treatment of malaria. Pasak Bumi is a commercial name for *Eurycoma longifolia* (Simarubaceae) plant species. Besides *Eurycoma longifolia* there are two other plant species also known locally as Pasak Bumi, *Rennelia elliptica* (Rubiaceae) and *Trivalvaria macrophylla* (Annonaceae). This study was performed to investigate the antimicrobial activities of the different species of Pasak Bumi and its total phenol contents. The antimicrobial activity of the ethanol extract was determined using the Agar Well Diffusion method at various concentrations while the phenol content was determined by the Folin - Ciocalteu method. The results of the ethanol extract from the different root showed that the *T. macrophylla* had the highest phenol content, and the highest activity index (AI) was found in the *E. longifolia* (0.96 at 1000 µg concentration). The results of this study show that the three different Pasak Bumi have potential as antimicrobials against oral pathogen; 1 yeast: *Candida albicans*, and 3 bacteria: *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus sobrinus*.

Keywords



Pasak Bumi, *Eurycoma longifolia*, *Trivalvaria macrophylla*, *Rennelia elliptica*, antimicrobial activity



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Author roles: **Kuspradini H:** Conceptualization, Data Curation, Formal Analysis, Methodology, Supervision, Writing – Review & Editing; **Silau S:** Formal Analysis, Investigation, Writing – Original Draft Preparation; **Supartini S:** Project Administration, Resources, Visualization; **Rosamah E:** Supervision, Visualization;

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Introduction

Pasak Bumi is a plant used in traditional medicinal that grows in the tropical forests of Kalimantan of Indonesia. It is used by the local people as an aphrodisiac, for postpartum treatment, fever, and malaria^{1,2}. In Central Kalimantan, there are three different plant species on Pasak Bumi; Yellow Pasak Bumi (*Eurycoma longifolia* Jack., Simaroubaceae), Red Pasak Bumi (*Rennelia elliptica*, Rubiaceae) and Black Pasak Bumi (*Trivalvaria macrophylla*, Rubiaceae)³. Previous research of Pasak Bumi (*E. longifolia* Jack) from different regions has shown activity in inhibiting the growth of microbes, however, research on the other species of Pasak Bumi such as *Rennelia elliptica* and *Trivalvaria macrophylla* are still limited. From the above information, the research aim is to compare the inhibition activities of the three different plants against one yeast: *Candida albicans*, and three bacteria: *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus sobrinus*. The research was also designed to extend our knowledge and help us explore the antimicrobial activities of the three different plant species.

Methods

Preparation of plant extracts

One kilogram of each plant was excavated and harvested from Katingan district, Central Kalimantan. The root was chopped and separated from its stem and leaves. The roots were sliced into small sections with a knife and allowed to dry under shade. The dried samples were crushed into powder using an electric blender. Once crushed, 50 grams of each powder of the plant root was weighed using a digital balance (Mettler Toledo, Mettler-Tokyo Group). Furthermore, the powder was extracted using successive maceration with the following solvents: *n*-hexane, ethyl acetate, and 96% ethanol. The ethanol filtrate was evaporated under a vacuum rotary evaporator (Eyela, N-N series) at 35°C until dry and used for the present study (Figure 1).

Total phenol content

The total phenolic content was determined spectrophotometrically (UV Mini 1240 Shimadzu) in accordance to the Folin-Ciocalteu method⁴. The sample solution was prepared by dissolving the dry extracts (2 mg) in 100 µl DMSO and 900 µl of distilled water. The reaction mixture was made by mixing 200 µl of the extract from sample solution (200 µg/mL), 300 µl of distilled water, 250 µl of 10% Folin-Ciocalteu reagent (Merck Millipore, CAS No. 109001) and 1250 µl of 7.5% sodium carbonate. After a 90 minutes incubation at room temperature, the absorbance was determined spectrophotometrically at 760 nm. Gallic acid (Wako, CAS No. 5995-86-8) was used as a reference standard for plotting a calibration curve (concentration range: 2 to 10 µg/mL). The total phenolic content was expressed as a Gallic Acid Equivalent (GAE)/mg extract, using a standard calibration graph.

Antimicrobial activity

Four pathogenic microbial strains; *C. albicans* (CA), *S. aureus* (SA), *S. mutans* (SM) and *S. sobrinus* (SS) from the Forest Product Chemistry Laboratory's culture collections, were used for the present study. The *in vitro* activity was screened using

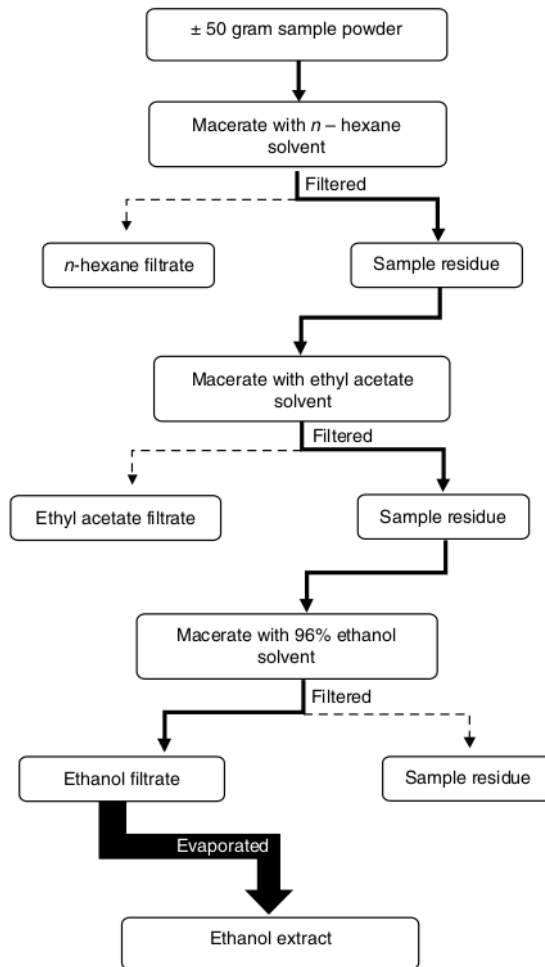


Figure 1. The extraction process of Pasak Bumi.

the agar well diffusion method in Nutrient Agar medium^{5,6}. The extracts of each plant at a concentration of 10 mg/ml in 40% ethanol were prepared, and an aliquot of the test solution was put in to get a final concentration of 100, 250, 500, and 1000 µg/well. It was then placed on the inoculated nutrient agar plates and incubated for ±18–24 h at 37°C. Ten µg/well of chloramphenicol (PT. Indofarma, Tbk., Indonesia) and 40% ethanol were employed as a positive and negative control. After incubation, the diameter of the inhibition zones was measured by a ruler. The experiment was performed in triplicate. The antimicrobial index (AI) was calculated using the formula^{6,7}: Activity index (AI) = Inhibition Zone of the sample/Inhibition Zone of chloramphenicol.

Statistical analysis

All experiments were conducted three times. Regression analysis was used to make a calibration curve and calculate the total

phenol content. All statistical analyses used Microsoft Excel 2010 software.

7 results

The total phenolic contents were calculated using the following linear equation based on the calibration curve of gallic acid: $y = 0.0667x + 0.009$; $R^2 = 0.9948$, where y is absorbance and x is amount of gallic acid in μg (Table 1). *T. macrophylla* root extract obtaining higher total phenolic content in

comparison to *E. longifolia* and *R. elliptica*. The extracts exhibited dose-dependent antimicrobial activities (Figure 2), and the results indicated that the *in vitro* antimicrobial activity of the *T. macrophylla*, *E. longifolia*, and *R. elliptica* extracts were ranked in the following order; $SS > SM > SA > CA$; $SA > SM > SS > CA$; and $SS > SA > CA > SM$, respectively. The highest activity was found in *E. longifolia* against *S. aureus*, with a maximum AI value (0.96) at 1000 $\mu\text{g/well}$ concentration while the lowest activity at all concentration was found in *R. elliptica* extracts.

Table 1. Total phenolic content from three different Pasak Bumi.

Sample		Calibration curve regression	Total Phenol ($\mu\text{g/mg}$ extract)
Scientific name	Local name		
<i>Trivalvaria macrophylla</i> (Blume) miq.	Black Pasak Bumi	$y = 0.0667x + 0.009$; $R^2 = 0.9948$	41.85 \pm 0.22
<i>Eurycoma longifolia</i> Jack.	Yellow Pasak Bumi		20.74 \pm 2.81
<i>Rennelia elliptica</i> Korth.	Red Pasak Bumi		4.37 \pm 0.57

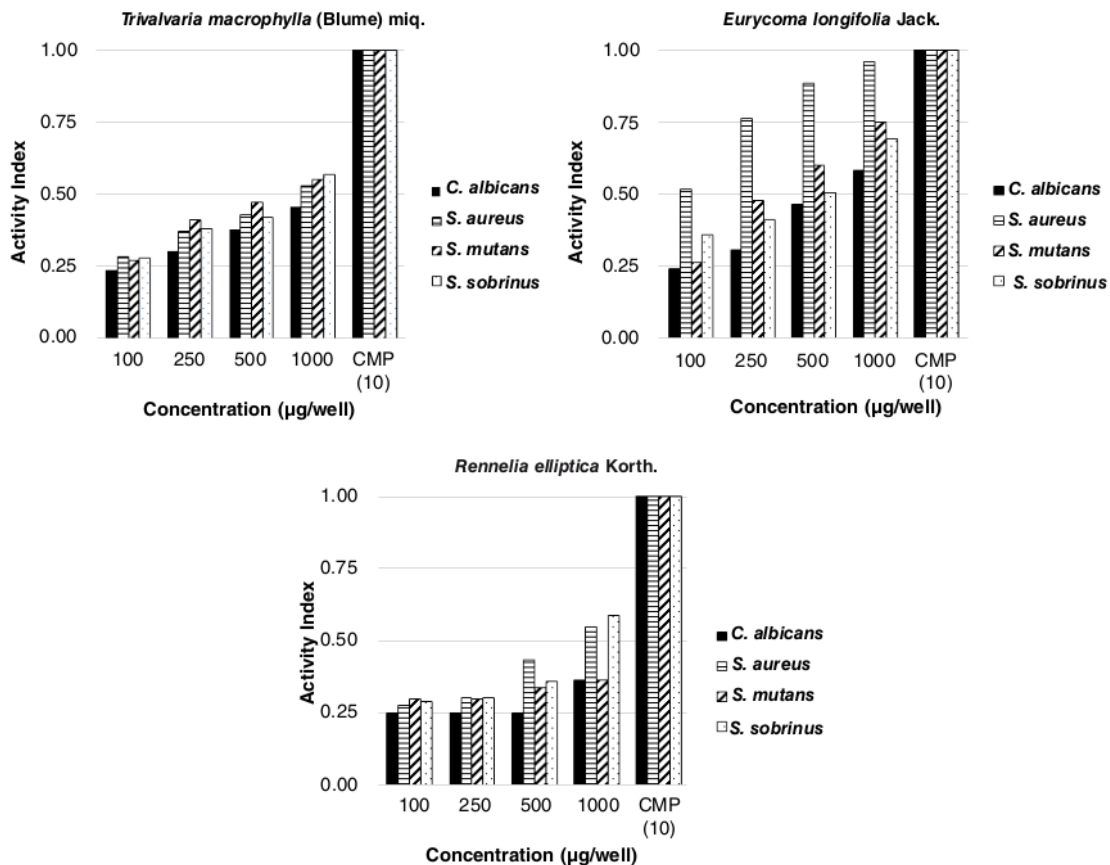


Figure 2. Antimicrobial activity Index of the three different Pasak Bumi.

Discussion

Plant extracts with a high AI value indicates that the extracts have good antimicrobial activity against the selected pathogens⁶. The inhibitory activity of *E. longifolia* root extracts was in agreement with previous literature, it could inhibit *S. aureus*⁸⁻¹⁰ and *C. albicans*¹¹. *R. elliptica* was found to be able to inhibit the growth of *C. albicans* and *S. aureus*, which is contrary to a previous study where it was found to be inactive¹²; however, there was no information about the extraction method for *R. elliptica* and the concentration used on that study. So far there have been no reports of the *T. macrophylla* being antimicrobial, but in this study *T. macrophylla* has proven to be an inhibitor for the growth of *S. aureus*, *S. mutans*, *S. sobrinus* and *C. albicans*. This is believed to be the first report to explore and compare the antimicrobial potentials of the three different Pasak Bumi plants. The antimicrobial activity may be attributed to the high content of the phenols present. Phenolic compound such as gallic acid can cause irreversible changes (such as charge, intra and extracellular permeability, and physicochemical properties) in the properties of microbial membranes, with consequent leakage of essential intracellular constituents¹³. *E. longifolia* possess a higher antimicrobial activity than *T. macrophylla*, but its phenolic content⁶ was lower than *T. macrophylla*. *E. longifolia* extract might contain more non-phenolic compounds, or possess phenolic compounds that contain a higher number of active groups than the other extract. The interactions between chemical compounds (phenolic and non-phenolic compounds) might also be responsible for the antimicrobial effects¹⁴.

Conclusions

The present study performed *in vitro* studies of antimicrobial properties of three different Pasak Bumi (*E. longifolia* Jack, *R. elliptica* and *T. macrophylla*) on oral pathogens which gave positive results and different degree of activity.

Data availability

Underlying data is available form Open Science Framework

OSF: Dataset 1. Pasak Bumi root extract, <https://doi.org/10.17605/OSF.IO/Q6X7R>¹⁵

License: CC0 1.0 Universal

Grant information

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² The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Lalit Sharma

Shoolini University, Solan, Himachal Pradesh, India

The manuscript presents an interesting study in which the antimicrobial activity of three plant species *Eurycoma longifolia* Jack., *Rennelia elliptica* Korth. and *Trivalvaria macrophylla* miq. was investigated.

Weakness of the manuscript

Introduction

1. Introduction part is very poor. The rationale of the need of comparative antimicrobial study of these plant species should have been elaborated.
2. No literature about the microbial infections has been cited. Authors needs to add some information about the microbial infections and about the role of herbal plants in treating microbial infections.
3. Authors must include the literature about the chief constituents present in these plant species.
4. Authors reported that Pasak Bumi is a local plant in Kalimantan, Indonesia. Authors must include other geographical regions where this plant grows.
5. Authors must include some information that why only *Candida albicans* yeast and *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus sobrinus* bacterial strains were taken for the study?

Methods

1. For identification of the phytoconstituents present in these plant species authors must have included the preliminary phytochemical screening, (a qualitative identification) or must have reported the literature on the phytochemical screening of these plants.
2. Why 40% ethanol was used to prepare different concentrations of the plant extracts?

3. In figure 1 authors have reported that ± 50 gram of sample powder was taken for further maceration with n-hexane. Is there any variations in the initial weight of the powder? Why symbol \pm is added? Is there any standard deviation or SEM?

Results

The activity index (Fig. 2) shows very little difference in concentration 500 and 1000 $\mu\text{g}/\text{well}$. As the concentration 1000 $\mu\text{g}/\text{well}$ is double of the 500 $\mu\text{g}/\text{well}$ so the difference in activity index should have been more. This suggests that something is wrong with the assays and/or with the presentation of the data. Authors need to analyze and discuss this point critically.

Suggestion/corrections in addition to the above

Grammatical errors/Corrections

Eg. In conclusion

The present study performed *in vitro* studies of antimicrobial properties of three different Pasak Bumi (*E. longifolia* Jack, *R. elliptica* and *T. macrophylla*) on oral pathogens which gave positive results and different degree of activity.

The above statement needs to be revised "The present study performed *in vitro* studies.....?"

Is the work clearly and accurately presented and does it cite the current literature?

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Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbial Infections, Natural Products

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 07 May 2019

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Sarifah Nurjanah 

Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Bandung, Indonesia

This is an interesting manuscript describing the antimicrobial activity of tree species plants ¹ known as **Pasak Bumi (*Eurycoma longifolia*, *Rennelia elliptica* and *Trivalvaria macrophylla*)** against bacteria: *Staphylococcus aureus*, *Streptococcus mutans* and *Streptococcus sobrinus* and yeast: *Candida albicans*. The study proved that Pasak Bumi not only can be used as an aphrodisiac, but also as a postpartum treatment for fever and malaria. It also has the potential as an antimicrobial agent. The paper is well written and structured, but there are some suggestions as follows:

Introduction:

1. In line 8, clarify the references of research on Pasak Bumi that have been done (references number 8, 9, 10 and 11).
2. In line 11 the authors said that there is no research on *R. elliptica* yet. This is not in accordance with what is written on the Discussion line 5-9 which states that there were studies on the antimicrobial activity of *R. elliptica* against *C. albicans* and *S. aureus*. So, it should be explained in the Introduction that there were antimicrobial studies on *R. elliptica* as well as *E. longifolia*.

Methods:

1. The authors used 760 nm wavelengths on the spectrometer in determining phenol content (used the Folin-Ciocalteu method), what is the reason for the use of these wavelengths? Do you use the results of other research or do you have your own tests? We recommend that you mention the basis used. Based on several studies there were also 750 nm (Rollando and Monica, 2018³) or 765 nm (Pourmorad *et al.*, 2006⁴) used.
2. It is not mentioned how long the maceration process was carried out for. We recommend that you write down how long the maceration process was for each solution (n-hexana, ethyl acetate and ethanol).
3. In determining the total phenol content, what DMSO stands for should be stated. Is it dimethyl sulfoxide?

Discussion:

1. The phenol component is thought to be a component that is responsible for antimicrobial properties. Although, the result showed that *T. macrophylla* contains higher phenol than *E. longifolia* but did not show higher antimicrobial activity. For this reason, it is better to find out its chemical composition to determine the components that affect antimicrobial activity.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: process engineering, essential oil, microbiology

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